Racemization of Optically Active Cysteine via Formation of 2,2-Dimethyl-4-thiazolidinecarboxylic Acid

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Synopsis. Optically active cysteine (Cys) was racemized via formation of (*RS*)-2,2-dimethyl-4-thiazolidinecarboxylic acid ((*RS*)-DMT) by refluxing in the presence of acetone in 10-fold molar amount in acetic acid. The formed (*RS*)-DMT was hydrolyzed by adding water to the reaction mixture to give (*RS*)-Cys in 95—97% yield.

(*R*)-Cysteine (abbreviated as (*R*)-Cys), a useful material for medicines, food additives, and cosmetics, has been obtained mainly from hair. Such acquisition of (*R*)-Cys from natural products is apt to be unstationary. (*S*)-Cys, a useful material for antibiotics, is difficult to obtain from natural products. An efficient synthesis of (*RS*)-Cys has been reported, [1,2] and (*R*)- and (*S*)-Cys can be obtained by an optical resolution using preferential crystallization.[3-5] (*R*)-Cys has been demanded in large amounts, but (*S*)-Cys is not in so large an amount. Therefore, a practical method for the racemization of optically active Cys must also be established to use the preferential crystallization procedure for industrial purposes.

Optically active amino acids are racemized in the presence of aldehydes in several organic acids, such as formic acid and acetic acid, and the racemization rate is largest in acetic acid.[6] However, Cys forms 4-thiazolidinecarboxylic acid derivatives by reacting with aldehydes or ketones.[7-9] Of these derivatives, 2,2-dimethyl-4-thiazolidinecarboxylic acid (DMT) is obtained by reacting Cys with acetone and easily hydrolyzed in water to regenerate Cys.[8,9] Threfore, the racemization of optically active Cys via formation of (*RS*)-DMT was tried in the presence of acetone in acetic acid.

Experimental

Materials (*R*)-Cysteine hydrochloride monohydrate was purchased from Wako Pure Chemicals Ind. and recrystallized from water. An aqueous solution of the hydrochloride was adjusted with concentrated aqueous ammonia to pH 6 to obtain (*R*)-Cys: $[\alpha]_D^{20}$ +6.49° (*c* 4.04, 5 mol dm^{-3} HCl) (lit,[10] $[\alpha]_D^{25}$ +6.5° (*c* 2, 5 mol dm^{-3} HCl)). (*S*)-Cys ($[\alpha]_D^{20}$ −6.52° (*c* 3.99, 5 mol dm^{-3} HCl)) was obtained by optical resolution using the preferential crystallization of (*RS*)-4-thiazolidinecarboxylic acid[4] or (*RS*)-cysteine salt of 4-ethylbenzenesulfonic acid.[5]

Racemization. A mixture of 0.0200 mol (2.42 g) of (*R*)- or (*S*)-Cys and 0.200 mol (11.6 g) of acetone in 30 cm^3 of acetic acid was refluxed (approximately 93 °C) for 1—2.5 h. After adding 10 cm^3 of water to the mixture, the solution was dried under reduced pressure at 45 °C and the residue was redissolved in 10 cm^3 of water. The solution was dried under reduced pressure at 45 °C. The suspension of the residue in 50 cm^3 of methanol was stirred for 30 min at room temperature. (*RS*)-Cys was collected by filtration, washed with methanol, and dried; the (*RS*)-Cys showed no optical rotation in water or 5 mol dm^{-3} hydrochloric acid having mp 222—225 °C (decomp). The ^{1}H NMR spectrum of the obtained (*RS*)-Cys in deuterium oxide was recorded on a JEOL JNM-PMX 60 NMR spectrometer, with no methyl protons indicated.

Synthesis of (*R*)-2,2-Dimethyl-4-thiazolidinecarboxylic Acid. A mixture of 0.0200 mol of (*R*)-Cys and 0.0400 mol of acetone in 30 cm^3 of acetic acid was stirred at 30 °C for 0.5 h. After evaporating the mixture to 5 cm^3 at 45 °C, 50 cm^3 of diethyl ether was added to the solution and the suspension was stirred for 4 h at room temperature. The formed (*R*)-DMT was collected by filtration and washed thoroughly with diethyl ether. Found: C, 44.53; H, 6.84; N, 8.55% (Calcd for $C_6H_{11}NO_2S$: C, 44.70; H, 6.88; N, 8.69%); yield 2.52 g (78.2%); mp 144—145 °C (lit,[8] 138—140 °C); $[\alpha]_D^{20}$ −188° (*c* 0.102, acetone) (lit,[8] $[\alpha]_D^{22}$ −183° (acetone)), $[\alpha]_D^{20}$ −144° (*c* 0.503, methanol).

Rate Constant of Racemization. A mixture of 0.0200 mol of (*R*)-Cys and 30 cm^3 of acetic acid was heated at 60, 70, 80, or 90 °C. After adding 0.100, 0.200, or 0.400 mol of acetone to the mixture, the resulting solution was stirred at each temperature; (*R*)-Cys was dissolved completely by stirring for several minutes. One cm^3 portions of the solution were pipetted out at appropriate time intervals and diluted rapidly to 10 cm^3 with acetic acid. The optical rotation at 589 nm was measured with a Union Giken PM-101 digital polarimeter equipped with a quartz cell of 0.50 dm path length. The rate constant of racemization (k_R/h) was calculated by the least-squares method from

$$\ln \alpha_0 / \alpha_t = k_R t, \quad (1)$$

where α_t is the optical rotation at time t h and α_0 that extrapolated to zero time. The half-life period ($t_{1/2}$/h) was calculated on the basis of k_R values obtained by Eq. 1.

The racemization rate for (*R*)-DMT was similarly measured at 90 °C by using 0.0200 mol of (*R*)-DMT and 0.180 mol of acetone.

Results and Discussion

Formation and Hydrolysis of (*R*)-2,2-Dimethyl-4-thiazolidinecarboxylic Acid. (*R*)-DMT was obtained in 78.2% yield by reacting 0.0200 mol of (*R*)-Cys with 0.0400 mol of acetone in acetic acid at 30 °C for 0.5 h, as described in the experimental section. In this reaction, (*R*)-Cys was completely dissolved by stirring for 20 min, and the resulting solution showed a negative optical rotation. If (*R*)-Cys is merely dissolved and (*R*)-DMT does not form, the solution should show a positive optical rotation since (*R*)-Cys has $[\alpha]_D$ +13.0° (acetic acid)[12] and (*R*)-DMT $[\alpha]_D^{20}$ −109° (*c* 0.103, acetic acid). This result indicates that (*R*)-DMT forms rapidly with dissolution of (*R*)-Cys. Optically active amino acids and their derivatives are racemized in the presence of aldehydes or ketones.[6,11]

The (*R*)-DMT obtained under the above conditions is optically pure because the specific rotation (−188 °) in acetone agrees closely with the reported value (−183 °).[8]

After stirring the above reaction mixture for 0.5 h at 30 °C followed by adding water, a drying of the solution gave (*R*)-Cys($[\alpha]_D^{20}$ +6.50 ° (*c* 3.97, 5 mol dm^{-3} HCl)) as the residue in 89.1% (2.16 g) recovery.

The above result indicates that (*R*)-DMT is formed rapidly by reacting (*R*)-Cys with acetone in acetic acid and is easily hydrolyzed by adding water to regenerate (*R*)-Cys.

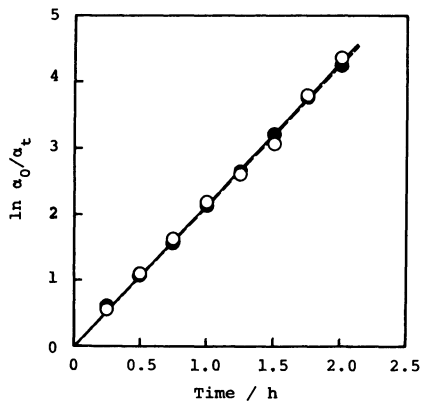

Fig. 1. First-order reaction for racemization.
Conditions: (*R*)-Cysteine ((*R*)-Cys) or (*R*)-2,2-dimethyl-4-thiazolidinecarboxylic acid ((*R*)-DMT) 0.0200 mol; acetic acid 30 cm^3; temperature 90 °C. —○—: (*R*)-Cys; acetone 0.200 mol. --●--: (*R*)-DMT; acetone 0.180 mol. α_0: Optical rotation extrapolated to zero time. α_t: Optical rotation at time *t* h.

Racemization of (*R*)-2,2-Dimethyl-4-thiazolidinecarboxylic Acid. (*R*)-Cys (0.0200 mol) was also reacted similarly with 0.200 mol of acetone at 50 °C for 1 h to give (*R*)-DMT in 85.9% (2.77 g) yield. The obtained (*R*)-DMT seemed to be partially racemized: $[\alpha]_D^{20}$ −139 ° (*c* 0.494, methanol); 96.5% optical purity. This result indicates that the formed (*R*)-DMT is racemized by increasing the amount of acetone and heating.

The optical rotation (α_t) of (*R*)-DMT or (*R*)-Cys was measured at 90 °C in the presence of acetone in 9- or 10-fold molar amount in acetic acid at 15 min intervals, as described in the experimental section. These racemizations can be regarded as first-order reactions because linear relationships are found between ln α_0/α_t and time *t*, as shown in Fig. 1. The rate constant (2.14 h^{-1}) for (*R*)-Cys agrees well with that (2.13 h^{-1}) for (*R*)-DMT. These results indicate that the rate-determining step is the racemization of (*R*)-DMT.

The rate constant for the racemization (k_R/h^{-1}) was measured at 60, 70, 80, or 90 °C. Acetone was used in 0.100, 0.200, and 0.400 mol for 0.0200 mol of (*R*)-Cys, the corresponding k_R's being represented as $k_{R(0.1)}$, $k_{R(0.2)}$, and $k_{R(0.4)}$, respectively. The k_R values and half-life periods ($t_{1/2}$/h) are listed in Table 1 for which the correlation coefficients between ln α_0/α_t and time *t* are over 0.99.

The $k_{R(0.2)}$ value (0.820 h^{-1}) at 80 °C is approximately equal to $k_{R(0.4)}$ (0.838 h^{-1}), though the $k_{R(0.1)}$ value (0.675 h^{-1}) is smaller than these values. Therefore, this racemization does not require acetone more than 10 times the molar amount of (*R*)-Cys. The dependence of $k_{R(0.2)}$ on temperature is given in the range 60—90 °C by

$$\ln k_{R(0.2)} = -12951 / T + 36.44, \quad (2)$$

Table 1. Rate Constant and Half-Life Period for Racemization[a]

Conditions		Rate constant	Half-life period
Temperature	Acetone	h^{-1}	h
°C	mol		
60	0.200	0.089	7.80
70	0.200	0.258	2.68
80	0.100	0.675	1.03
80	0.200	0.820	0.85
80	0.400	0.838	0.83
90	0.200	2.14	0.32

a) (*R*)-Cysteine (0.0200 mol) and acetone were stirred in 30 cm^3 of acetic acid.

Table 2. Racemization of Optically Active Cysteine[a]

Configuration of optically active Cys	Racemization period	Yield of (*RS*)-Cys
	h	g [%[b]]
(*R*)	1.0	2.32 [95.7]
(*R*)	1.5	2.33 [96.0]
(*R*)	2.0	2.34 [96.7]
(*S*)	2.0	2.35 [96.9]
(*S*)	2.5	2.35 [96.9]

a) Cys: Cysteine. Conditions: (*R*)- or (*S*)-Cys 0.0200 mol; acetone 0.200 mol; acetic acid 30 cm^3; temperature approximately 93 °C. b) The yield was calculated on the basis of the amount of (*R*)- or (S)-Cys used by reaction.

where T/K is temperature, and the correlation coefficient has been found to be 0.999. The $k_{R(0.2)}$ value at 50 °C was calculated to be 0.0262 h^{-1} from Eq. 2, which value suggests that the (*R*)-DMT obtained from the reaction at 50 °C for 1 h has 97.4% optical purity. The calculated optical purity agrees well with that (96.5%) of the obtained (*R*)-DMT.

Racemization of Optically Active Cysteine. Based on the above results, (*R*)- or (*S*)-Cys was racemized in the presence of acetone in 10-fold molar amount under reflux (approximately 93 °C). The results are listed in Table 2.

The k_R and $t_{1/2}$ values at 93 °C were calculated from Eq. 2 to be 2.90 h^{-1} and 0.24 h, respectively. These values suggest that 99.7% of optically active Cys is racemized by reacting for 2 h. As seen in Table 2, the racemization is almost complete in reaction for 2 h, and (*RS*)-Cys is obtained in high yield (97%).

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